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Cytogenetical and Biochemical Researches in the Rumanian Hamster *(Mesocricetus newtoni)*

By P. Raicu, M. Hamar, S. Bratosin and I. Borsan

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Introduction

Nehring (1898) considered the Rumanian hamster *Mesocricetus newtoni* to be a good species but subsequently it was regarded as a subspecies of the Syrian hamster *(Mesocricetus auratus)*.

The taxonomic, biological and ecological investigations of Marches (1964) supported the earlier view as did the ecological work of Hamar and Sutova (1966) on this and related species. Raicu and Bratosin (1966) have shown that *Mesocricetus newtoni* is chromosomally different from the Syrian hamster.

We have examined mitosis and meiosis in *Mesocricetus newtoni* and undertaken a comparative study of proteins and free amino-acids in the serum and liver of this and other hamsters.

Material and methods

Animals from the Department of Genetics (University of Bucharest) and from Department for rodents (Agricultural Institute of Bucharest) have been used for this study. For cytological study, the animals were injected with 0.06% colchicine for two hours. Bone marrow and testes was treated with a hypotonic solution of natrium citrate and fixed with alcohol-acetic acid (3,1). Staining was performed in Giemsa solution.

A comparative electrophoretic study from the proteins of the blood serum was performed. Proteins have been separed electrophoretically on Whatman paper no. 1 with veronal-natrium veronal buffer solution (pH = 8.6). The proteic fractions have been evidenced with a blue brom-phenol solution and quantitative determination was performed by the Opton densitometer.

The free aminoacids in blood and liver were studied by paper chromatography. The material was treated in 0.04 N acetic acid at 100° C for two minutes. The proteins were precipitated and eliminated by centrifugation. The supernatant was used for chormatographical purpose, having as solvent n — butanol — acetic acid — bidistilled water (4:1:5). The free aminoacids have been identified with a 0.2% ninhidrin solution in n — butanol and by means of control aminoacids.

Results and discussions

The eighteen pairs of autosomes of *Mesocricetus newtoni* (2n = 38) consist of 2 metacentrics (1 and 9), 5 submetacentrics (2, 8, 14, 16 and 17) and 11 subtelocentrics (3—7, 10—13, 15 and 18). The heterosomes in females are represented by a pair of X chromosomes which are the longest subtelocentrics. In males the XY heterosomes are different, the Y chromosome being the smallest submetacentric (fig. 1 and 2).

A comparison of the complements of the Syrian (2n = 44) and Rumanian (2n = 38) hamster suggests that the absence of acrocentric chromosomes in the latter is due to a centric fusion. Ecological evidence indicates that the Syrian hamster is the older species. The authors consider that the speciation in this case is due to a robertsonian process of centrical fusion and the translocation of a pair of chromosomes. This is also suggested by the modification of heterosomes.

Fig. 1. Female karyotype in *Mesocricetus newtoni* (left) and their metaphase plate (right)

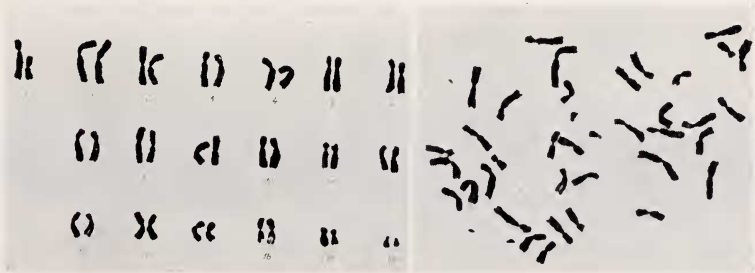

Fig. 2. Male karyotype in *M. newtoni* (left) and their metaphase plate (right)

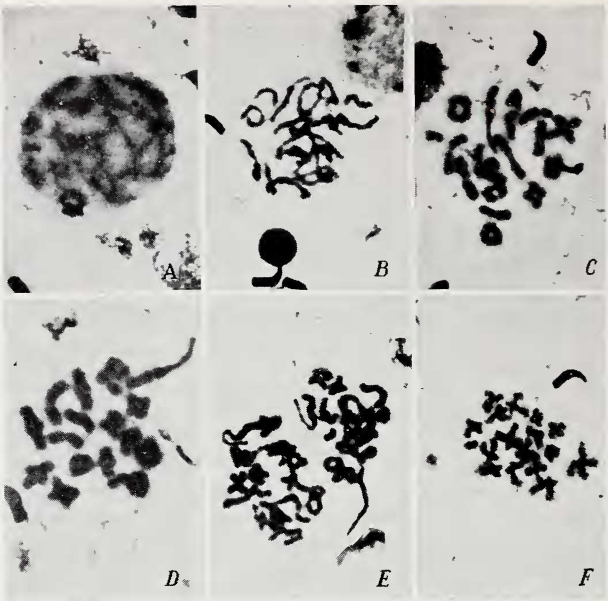

Fig. 3. Male meiosis in *M. newtoni:* leptonem (A), diplonem (B), methaphase I (C, D, E,) methaphase II (F)

The karyotype of *M. newtoni* (Raicu and Bratosin, 1966) reveals some differences, because formerly the investigations have been carried out only on few animals and on strongly colchicinized metaphases. This is a correction of our first study on the karyotype in the Rumanian hamster.

The sex chromosomes are heteropycnotic at leptotene in the male (fig. 3, A). By diplonem (fig. 3, B) the sex vesicle was completely disappeared and the heterosomes can be clearly distinguished. They form the longest of the 19 bivalents at metaphase I (fig. 3, C, D, E).

The study of 10 metaphasis cells (fig. 4) has shown that ring bivalents vary between 2—4, cross bivalents between 4—8 and the rod bivalents between 7—12. We have found a constant end-to-end association between the short arm of the Y chromosome and the short arm of the X chromosome. The long arm of the X chromosome is less contracted and isopycnotic, consequently it is less stained than the other part of the bivalent. Thus, in structure, staining and association the sex chromosomes of the Rumanian hamster differ from those of *M. auratus* (2n = 44), *Cricetus cricetus*

Average quantity of various blood serum proteic fractions

(in %)

Species	Albumins	Globulins				
		α_1	α_2	β_1	β_2	γ
Mesocricetus auratus	44.0	11.0	13.5	7.0	14.5	10.0
Mesocricetus newtoni	44.5	14.0	9.2	6.3	12.5	13.5
Cricetus cricetus	45.5	15.4	12.7		11.8	14.5

(2n = 22) and *Cricetulus griseus* (2n = 22) (Fredga and Santesson 1964) (fig. 5). In *Cricetulus griseus* and *Cricetus cricetus* species there is a partial homology of the short arms of X and Y chromosomes, that is why they associate side-by-side. The long arms of the X chromosomes in the Rumanian and Syrian hamsters are isopycnotical and different in size; the arms of the X chromosome in *M. newtoni* are much longer than those of *M. auratus*.

Fig. 4. Metaphase I in male meiosis of *M. newtoni*

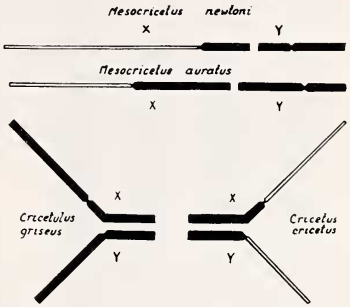

Fig. 5. Schematical illustration of the sex bivalents in four hamster species (modified after K. Fredga and B. Santesson, 1964)

The mitotical and meiotical study on the Rumanian hamster as compared with other hamster species have shown considerable differences not only in the number of chromosomes, but also in their structure, homology and chiasmata. This phenomenon is very obvious in heterosomes. By comparing the karyotypes of these 4 hamster species, it resulted that the Rumanian and Syrian hamsters are closely related.

Comparative electrophoretical researches of the blood serum in *M. newtoni, M. auratus* and *Cricetus cricetus* have shown that the albumin fraction as well as the 5 globulinic fractions (α 1, α 2, β 1, β 2 and γ) show considerable differences (table). Thus these species are biochemically well individualized, in spite of their partial homology of proteic fractions. This homology proves their phyllogenetic link (fig. 6, A, B, C).

Chromatographic study of free aminoacids in the blood serum of *M. auratus* (fig. 7, a) and of *M. newtoni* (fig. 7, a') reveal considerable differences between these two species. Thus there is a much higher quantity of valine in the Syrian hamster than

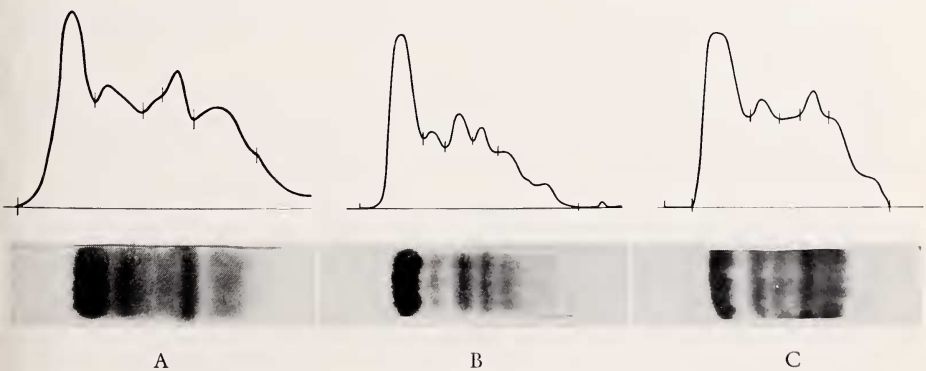

Fig. 6. Electrophoriograms in *Mesocricetus newtoni* (A), *Mesocricetus auratus* (B) and *Cricetus cricetus (C)*

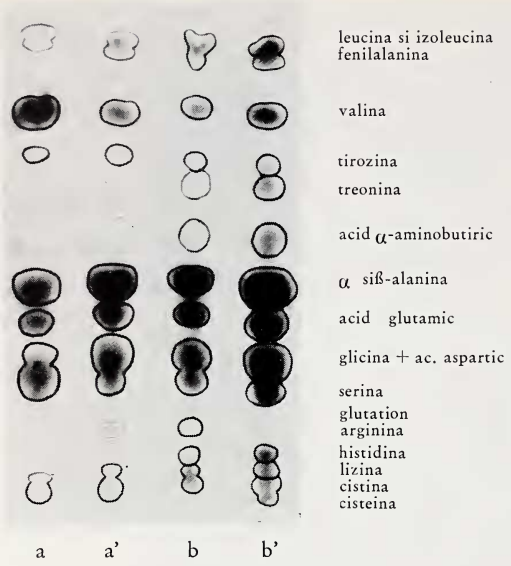

Fig. 7. Chromatograms of free aminocids from the serum blood in *M. auratus* (a), *M. newtoni* (a') and from the liver in *M. newtoni* (b) and *M. auratus* (b')

in the Rumanian one. α-Aminobutiric acid is absent in the Syrian hamster and scarcely to be found in the Rumanian one, while α and β alanine, glicine and the aspartic acid are in higher quantities in *M. newtoni*. In the Rumanian hamster we found only small quantities of arginine and traces of histidine, while in the Syrian hamster these aminoacids are completely missing.

The quantities of free aminoacids in the liver of the Rumanian hamster (fig. 7 b) and in the Syrian hamster (fig. 7 b') are variable. Thus higher amounts of leucine, phenilalanine, valine, α-aminobutiric acid, α and β alanine, glutamic acid, glicine, aspartic acid and serine are recorded in the latter. Arginine is present only in *M. newtoni*, and cisteine only in *M. auratus*.

Electrophoretic and chromatographic studies of proteic fractions and of free aminoacids on the blood serum and the liver in the three hamster species proves biochemical differences typical for each species. In the speciation process besides chromosomal mutations which have determined the formation of different karyotypes, there have also taken place many genic mutations producing biochemical differences. Our cytogenetical and biochemical studies prove that the Rumanian hamster is a species in itself, with its own morphological, ecological, genetical and biochemical characteristics. Presently we intend to obtain inbreeding in order to turn this species into a laboratory animal.

Conclusions

1. The Rumanian hamster is cytogenetically different from other hamster species, particularly in regard to the number and structure of chromosomes and the homology and pairing of the sex chromosomes.
2. Comparative biochemical studies have shown various differencies between the Rumanian and the other hamster species, as for instance the number and the quantity of proteic fractions, as well as the variable quantity of free aminoacids in blood serum and liver.
3. In the Rumanian hamster the speciation process occurred by many chromosomal and genic mutations, which have determined a karyotypical and biochemical individuality. The Rumanian hamster is more closely related to the Syrian hamster, than to other hamster species.

Summary

In the Rumanian Hamster *(Mesocricetus newtoni)* the karyotype is represented by 18 pairs of autosomes, i. e. 2 pairs metacentric, 5 submetacentric and 11 subtelocentric. In females the sex chromosomes (XX) are the biggest subtelocentric ones, while in males (XY) the Y chromo-

some is the smallest submetacentric one. By comparing the reductional division (meiosis) in *Mesocricetus newtoni* (2n = 38), *M. auratus* (2n = 44), *Cricetus cricetus* (2n = 22) and *Cricetulus griseus* (2n = 22) considerable differences in structure, stainability, way of associating the arms of sex chromosomes have been found in the Rumanian hamster as compared to the other species.

Comparative biochemical researches have proved that between the Rumanian hamster and the other hamster species there are quite a lot of differences in the number and amount of the proteic fractions in blood serum as well as in the free aminoacids in the blood and liver.

Based on the comparative study of the karyotypes in the Rumanian and the Syrian hamster as well as according to ecological investigations which have shown that the Rumanian hamster is an older species, the authors consider that the karyotype differences in the Rumanian hamster are the results of a Robertsonian centrical fusion process and the translocation of pair of chromosomes. Gene mutations have then followed contributing to the phenotype differentiation and to establishing a definitive biochemical individuality.

Zusammenfassung

Beim rumänischen Hamster *(Mesocricetus newtoni)* ist der Karyotypus von 18 Autosomenpaaren vertreten, und zwar sind davon 2 metazentrisch, 5 submetazentrisch und 11 subtelozentrisch. Bei den Weibchen sind die größten Geschlechtschromosomen (XX) die subtelozentrischen, während bei den Männchen (XY) die Y Chromosome die kleinste submetazentrische ist. Vergleichende Untersuchungen der Reduktionsteilung (Meyosis), die bei *Mesocricetus newtoni* (2n = 38), *M. auratus* (2n = 44), *Cricetus cricetus* (2n = 22) und *Cricetulus griseus* (2n = 22) durchgeführt wurden, zeigten erhebliche Unterschiede zwischen dem rumänischen Hamster und den übrigen Arten in bezug auf Struktur, Anfärbung und Zusammenfügung der Arme der Geschlechtschromosomen.

Durch vergleichende biochemische Untersuchungen wurde nachgewiesen, daß zwischen dem rumänischen Hamster und den übrigen Arten eine Reihe von Unterschieden in Hinsicht sowohl auf Anzahl und Menge der Eiweißfraktionen im Blutserum als auch auf die freien Aminosäuren im Blut und in der Leber vorhanden ist.

In Anlehnung an vergleichende Untersuchungen der Karyotypen beim Hamster aus Rumänien und aus Syrien sowie auch an ökologische Forschungen, deren Ergebnisse darauf hinweisen, daß der in Rumänien vorkommende Hamster eine ältere Art ist, vertreten die Verfasser die Ansicht, daß die karyotypischen Unterschiede des Hamsters aus Rumänien auf einem Robertsonischen zentrischen Fusionsprozeß und auf der Translokation eines Chromosomenpaares beruhen. In der Folge gesellte sich auch eine Genmutation dazu, die zur phänotypischen Differenzierung und zu einer endgültigen biochemischen Individualität ihren Beitrag brachte.

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SCHRIFTENSCHAU

Flade, Johannes Erich: **Das Araberpferd.** 2. neubearbeitete Auflage. Die Neue Brehm-Bücherei, 291, A. Ziemsen-Verlag, Wittenberg-Lutherstadt 1966. 119 S., 60 Abb. 8,— DM.

Nur 4 Jahre nach Erscheinen der 1. Auflage liegt jetzt die 2. Auflage des Buches von Flade über das Araberpferd vor. Der Autor hat in dieser Schrift die wichtigsten historischen Daten über das Araberpferd und seine Zucht zusammengestellt und unterrichtet über die strukturellen und physiologischen Merkmale dieser Vollblutrasse. Das Kapitel über Herkunft und Zuchtgeschichte ist weitgehend neu gestaltet worden. Auch an anderen Stellen des Textes sind Verbesserungen und Ergänzungen vorgenommen worden. Der Text wird wie in der 1. Auflage durch zahlreiche Tabellen und graphische Darstellungen ergänzt. Das Buch kann jedem, der sich über Eigenschaften und Geschichte des Arabischen Vollblutpferdes orientieren will, empfohlen werden.

H. Bohlken, Kiel

Goerttler, Victor: **Neufundländer.** Die Neue Brehm-Bücherei Nr. 371. A. Ziemsen-Verlag, Wittenberg-Lutherstadt 1966. 144 S., 87 Abb. 10,— DM.

Ein erfahrener Biologe und begeisterter Hundefreund hat mit diesem Werk ein erstklassiges Hundebuch vorgelegt, welches weit mehr als nur einen Bericht über den Neufundländer gibt. In klarer Form wird das jetzige Wissen über die Abstammung des Haushundes und die Herausbildung seiner Rassen, alten aber unbewiesenen Annahmen über diese Fragen gegenübergestellt. Einem sorglichen Bericht über die Entwicklung des Neufundländers und seine Verbreitung in verschiedenen Ländern folgt eine treffliche Kennzeichnung von Verhaltenseigenarten. Hinweise auf Zucht, Fütterung und Verwendung der Neufundländer beschließen das Buch.

W. Herre, Kiel

Larsen, May und Henry: **Durch Gottes Zoo.** Erlebnisse mit Tieren und Menschen in Mittelamerika, Guayana, den Antillen und Neukaledonien. Albert Müller Verlag, Rüschlikon-Zürich — Stuttgart — Wien 1966. 216 S., mit zahlreichen Federzeichnungen, 4 farbigen, 35 Schwarz-Weiß-Tafeln. 22,80 DM.

Die Autoren, May und Henry Larsen, erzählen in diesem Buch von ihren langen Reisen in die reizvolle Urwald- und Inselwelt Südamerikas und der Südsee. Die liebevollen und lebendigen Schilderungen der Tiere, die sie als Präparatoren beobachtet und gesammelt haben, und der vielseitigen Begegnungen mit den Menschen dieser Länder zeugen von Einfühlungsgabe und Verstehen, die doch auf nüchterner Beobachtung und jahrzehntelanger Beschäftigung basieren.

Die heitere Episode aus dem Leben eines Kolibripärchens, Percinet, der koboldhafte Tukan, und der Trompetervogel als Hühnerhirte erscheinen ebenso lebendig wie der kleine, anhängliche Schimpanse Julot, die Faultiere, Ameisenbären, Gürteltiere, das Tapir-Junge, Kiki, der Klammeraffe und die Vampire, die überall ihr grausames Unwesen treiben.

Ein rechtes Buch für den Tierfreund!

W. Kleffner, Hannover